# 1998 Runme Shaw Memorial Lecture: Somatic Evolution of Cancer

W F Bodmer,* *PhD, FRCPath, FRS*


## Abstract

***The interpretation of cancer as a somatic evolutionary process involving genetic mutation followed by selection, traces its origins to the early years this century. The dramatic developments in molecular genetics have substantiated these early ideas. Through the application of positional cloning and genomic analysis, many mutations in particular genes, both dominant oncogenes and tumour suppressor genes have now been found in a wide variety of tumours. Other genetic events such as non-disjunction leading to haploid expression of a gene and so reduced gene dosage, or epigenetic changes following, for example, changes in methylation patterns leading to reduced orincreased gene expression, may also play critical roles in the progression of a cancer.***

***The analysis of mutations at different stages of colorectal cancer provides a good model for following the initiation and progression of a cancer. Mutations in the APC gene, which explain familial adenomatous polyposis, occur in a high proportion of sporadic colorectal carcinomas and appear to be the earliest known changes. Patterns of mutation in the gene suggest dominant negative or gain of function effects, and also reveal important lowpenetrance subpolymorphic missense mutations that nevertheless may have a very significant impact on the genetic contribution to colorectal cancer susceptibility. Mutations are also found in related genes in the APCpathway,such as β-catenin and E-cadherin. Mutations in mismatch repair genes (hMLH1 and hMSH2) have also been shown to occur, as well as reduced expression due to methylation changes, in 10% to 20% of sporadic colorectal carcinomas. In addition, mutations in the well known oncogenes p53 and ras are commonly found.***

***Thegrowth of a cancer is a balance between the rate of cell division and the rate of cell death or apoptosis. Thus, genetic changes which reduce theprobability of apoptosis, such asp53 andprobably hMLH1, are as important a feature of the evolution of a cancer asthose which enhance the independence (APC) and rate of cell division (growth factors). Simple models for the evolution of a cancer that take into account these two processes, show that cancers evolve initially by a series of finite increases in cell population size, following which there may be long periods of cell turnover during which there is an opportunity for further mutation and selection. This explains the long lag periods between the initiation and subsequent progression of most cancers.***

***Our rapidly developing understanding of cancers at the fundamental genetic level provides new opportunities for developing targeted treatments, as well as novel approaches to prevention and early detection.***


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***Key words: APC, Apoptosis, Colorectal, Mismatch repair, Mutation***

## Introduction

The interpretation of cancer as a somatic evolutionary process involving genetic mutation followed by selection, goes back to the early years of this century. Boveri's[1] hypothesis put forward in 1914 that cancer was associated with abnormalities of the chromosomes and Tyzzer and Strong's[2] experiments of transplantable tumours in 1916 already established the notion of a somatic evolutionary basis for cancer.

The dramatic developments in molecular and cellular genetics over the last quarter of century have firmly substantiated these fundamental ideas about the nature of cancer. The aim of this paper is to illustrate these notions with examples taken particularly from work on colorectal cancer, which has provided an excellent model for following the initiation and progression of cancers.

## Genetic Changes in Cancer

The first clear-cut evidence for a specific chromosomal change in a cancer was the discovery in the early 1960s by Nowell and Hungerford[3] of the Philadelphia chromosome in chronic myelogenous leukaemia (CML). Following the interpretation by Janet Rowley[4] of the Philadelphia chromosome as a translocation between chromosomes 9 and 22, the nature of the specific genetic change at the breakpoints was identified by molecular technique. This depended on the discovery by Varmus

* *Head*
*Cancer and Immunogenetics Laboratory*
*Institute of Molecular Medicine, Oxford, England*
*Address for Reprints: Sir Walter F Bodmer, ICRF Cancer and Immunogenetics Laboratory, Institute of Molecular Medicine, Oxford OX3 9DS, England.*

and Bishop and others of the dominant oncogenes carried by the oncogenic retroviruses first identified by Peyton Rous in 1910 (see Franks and Teich,[5] for the general background and references to these ideas). The dominant oncogenes are identified as extra sequences which are altered versions of normal genes present in normal host cells, but carried by the retroviruses. These sequences enable them to transform even in the presence of the normal versions in the host cells that they invade, which establishes their dominance. It is one of these dominant oncogenes that is essentially created by the Philadelphia chromosome translocation. In many cases, it has been shown that these sequences can become dominant oncogenes in a cell, without the involvement of a virus, by conventional genetic mechanisms of mutations. One such dominant oncogene, K-ras, which carries out a key signalling function in the cell, has been shown to be frequently mutated in many human cancers, including colorectal cancer.[6]

A different class of genetic changes in cancers that are fundamentally recessive in their mode of action was suggested by Knudson in 1971.[7] He pointed out that, if cancer arises through a series of somatic, genetic changes, then sometimes one of those genetic mutations may be inherited through the germline and so be present in every cell in the body. Such individuals, therefore, have cells that have already taken one step along the genetic pathway leading to a cancer and that can be the basis of a dominantly inherited cancer susceptibility. Knudson further suggested that an additional genetic step was needed at the somatic level in order to knock out the activity of the remaining normal gene. Thus, the familial inherited susceptibility is dominant, but the second genetic event that occurs somatically in the progressing cancer means that, at the somatic level in the cancer, the genetic effect is recessive. These ideas thus predicted a class of genetic changes in tumorigenesis whose normal functions were a block to the development of a cancer, and where recessive genetic change knocking out their function was the positive step contributing to the progression of a cancer. Such genes have sometimes also been called tumour suppressor genes. Knudson's ideas suggested that finding genes for dominant inherited cancer susceptibilities might therefore lead to the discovery of key recessive or tumour suppressor genes relevant to the somatic evolutionary development of a sporadic, non-inherited cancer.

**Familial Adenomatous Polyposis: A Model for Tumour Suppressor Genes**

Familial adenomatous polyposis was first described in the late 19th century but was shown by Lockhart-Mumme at St. Mark's Hospital (with which I and my colleagues have collaborated for many years) in the 1920's to be a clearcut Mendelian dominantly inherited colorectal cancer susceptibility (see Bodmer and Wasan in Franks and Teich[5]). Affected individuals usually develop from a few hundred to several thousand adenomatous polyps in the colon and rectum starting in their early teens which, if left untreated, inevitably give rise to one or more colorectal carcinomas in the third or fourth decade. Total colorectomy removes the major part of this risk if carried out sufficiently early, though leaving the risk of certain other manifestations, including upper GI tract cancers and desmoids which may be a serious cause of later mortality.

The clue to finding the position of the relevant gene, APC, came from a case report by Herrera and colleagues[8] who identified an individual with polyposis and other complications which led to the identification of a deletion of chromosome band 5q21. This clue led to the mapping of the APC gene, by now standard DNA techniques, to position 5q21[9] and so using positional cloning techniques to the identification of the APC gene itself.[10,11]

The APC gene codes for a 2843 amino acid protein which has been shown to be mutated in the vast majority of cases of familial adenomatous polyposis.[12,13] More than 95% of the mutations found in this way are either nonsense or frameshift mutations which lead to a truncated protein product effectively knocking out the function of the gene. Furthermore, in confirmation of Knudson's ideas, up to 70% or 80% of sporadic colorectal cancers also have such truncating mutations.

Intriguingly, mutations are only found in a region extending approximately from amino acid 200 to 1600. Mutations, even if truncating outside this region of the gene, do not seem to have a sufficiently severe effect on the function of the APC protein to give rise to classical florid adenomatous polyposis. Mutations in the 5' end of the gene give rise to an attenuated form of polyposis with smaller numbers of polyps and later onset of cancers[14] and mutations in the 3' segment of the APC gene mav do likewise.[15]

This is also a striking difference between the distribution of somatic as compared to germline mutations. The germline APC mutations are distributed more or less uniformly throughout the relevant first half of the APC gene, apart from a few obvious mutational hotspots,[16] while some 60% of the somatic mutations cluster in a region around the amino acids 1200 or 1300 to 1500 or 1600.[12] The surprising phenotypic variation caused by truncating mutations, which should apparently lead to the same phenotype, can to a large extent, be explained by the fact that the protein product functions as a dimer and that heteropolymers formed of mutant and normal forms of the protein may vary in their level of activity from near normal to almost completely negative. Thus, for example, mutations in the 3' end of the gene beyond

amino acid position 1600 presumably give rise to heteropolymers with normal or near normal function, and hence at most minor defects such as an increased tendency to multiple polyps.

Recently, an interesting new class of APC mutations has been described. These are missense mutations which appear to increase the risk of colorectal tumours or give rise to multiple adenomas but without the high penetrance that gives rise to the typical Mendelian familial adenomatous polyposis family.[17,18] It appears that there may exist in the population variants of the APC gene at subpolymorphic frequencies which have a significant but imperfectly penetrant effect on the incidence of colorectal adenomas and carcinomas. Such mutations would be associated with little or no selective disadvantage and therefore can easily increase in a population due to random genetic drift. In some cases, as for the I1307K mutation found in Ashkenazi Jewish populations, the variants may reach relatively high and obviously polymorphic frequencies, in that case between 6% and 8%. The variety of APC variants that may exist in a population and be associated with significant risk of colorectal carcinoma, as well as the fact that 30% to 40% of flank familial adenomatous polyposis will arise in individuals carrying new mutations, suggests that there is a case for offering population-wide screening for APC mutations, since in all these situations regular screening for adenomas by colonoscopy, followed by their removal if found, can essentially remove the risk of such APC variant carriers getting colorectal cancer.

The DNA sequence changes found in somatic APC mutations in sporadic colorectal cancers include a mixture of predominantly small deletions and a variety of single basepair changes.[19] The pattern of sequence changes, which includes only a minor component of G-A point mutation transversions, clearly does not indicate a major role for external mutagens in the genesis of colorectal cancers.[20] This is particularly significant since a number of lines of evidence indicate that APC mutations, when they occur, are probably the earliest events in the initiation of a colorectal cancer. It is at this stage that mutagenesis is most critical and therefore that the effects of external mutagens would be most obvious. The p53 gene, which is probably the most commonly mutated gene found so far in all human tumours, and is mutated in approximately 50% of colorectal carcinomas, supports this view.[21] Thus, essentially no p53 mutations in colorectal carcinomas are G-A transversions, while these form 40% or more of the p53 mutations found in lung cancer, just as expected from the mutagenic effects of the hydrocarbons in cigarette smoke. These data strongly suggest that environmental effects on colorectal cancer commonly associated with diet must be of a broadly "promotional" rather than mutagenic nature.

While the protein sequence of the APC gene product did not at first give any clues as to its function, more recent studies have shown that it is involved in a complex of molecules that play a critical role in the control of cell-cell and also most probably cell basement membrane attachment.[22,23] A proximal function of the APC protein appears to be to control the turnover of β-catenin which itself can act both as a control of the epithelial adhesion molecular, E-cadherin, as well as a signalling molecule to the nucleus associated with growth stimulation. Mutations in the β-catenin gene have been found in up to 25% of colorectal carcinomas.[24,25] Some of these mutations appear to be found particularly in colorectal carcinomas which do not have APC mutations. The region of the APC gene where somatic mutations are clustered, and which includes the two missense mutations with low penetrance effects on colorectal adenomas and carcinomas, is a region of the protein that may be most critical with respect to interactions with β-catenin and other relevant proteins. Thus, mutations in this part of the molecule may have relatively strong dominant negative effects and so more extreme functional consequences, which could explain both the mutation cluster region and the fact that missense mutations in this region involving charge changes may have more significant effects than other mutations elsewhere.[18]

The overall data clearly indicate that interference with cellular attachment, and the consequent derangement of cellular architecture and probable encouragement of independence of growth, are key early steps in the development of a tumour and account for the importance of early mutations in the APC and probably β-catenin genes in colorectal carcinomas.

The multiple intestinal neoplasia (MIN) mouse carries a nonsense mutation in the mouse version of the APC gene which, as in the human situation, gives rise to intestinal tumours.[26] Although there are significant differences between the mouse and human situations, with the former having most of its tumours in the small intestine and dying of intestinal obstruction before the development of frank carcinomas, the MIN mouse is nevertheless a valuable model for the experimental study of the control of human colorectal tumorigenesis. It has, for example, been possible to show in MIN mice the direct effect of increasing fat in the diet on increasing the number of tumours.[27] This effect appears to depend on the microbial flora in the intestine and strongly suggests that it is the interaction between fat in the diet and its metabolism by the gut microflora that contributes to the development of human colorectal carcinomas. Combining the MIN mutation with mouse germline versions of other mutations known to occur somatically in human colorectal carcinomas should make it possible

to provide an appropriate model of spontaneous tumorigenesis in the mouse. This should surely then provide the best basis for the study of both environmental factors in a cancer, as well as testing novel therapies.

**Hereditary Non-Polyposis Colorectal Carcinomas (HNPCC) and Mismatch Repair or Replication Error Mutations**

Families with multiple cases of cancers indicating a dominant pattern of inheritance, were first clearly described by Henry Lynch. Because colorectal cancer was relatively common in these families, and they were clearly distinguished from familial adenomatous polyposis, they have been described as hereditary non-polyposis colorectal cancer families or HNPCC. Following the localisation of genes for HNPCC families to chromosomes 2 and 3, and the identification of a replication error phenotype, represented by somatic mutations in CA repeat positions, in colorectal carcinomas from affected individuals, mismatch repair genes hMSH2 and hMLH1 were identified respectively on chromosomes 2 and 3 and mutations in them account for the majority of HNPCC families.[28-30] The normal cells of HNPCC individuals carrying mutations in these mismatch repair genes, but in the heterozygous state, do not show an increased mutation rate. The tumours which occur in HNPCC individuals do have increased mutation rates at repeat sequences, which are particularly prone to mismatch repair, and so as in the case of classical tumour suppressor genes, must involve a second mutational event that removes the remaining normal activity since the mismatch repair defect is clearly recessive at the cellular level.

Approximately 15% of sporadic colorectal carcinomas, and a varying percentage of other human cancers, have a mismatch repair phenotype that can, in some cases, only be associated with mutations in one or more of the mismatch repair genes. Tumours with the mismatch repair defect frequently have mutations in the HLA associated protein β2-microglobulin, most probably because of selection for escape from immune response to the many bystander mutations that may be created as a result of the mismatch repair defect during colorectal carcinogenesis. Lack of, or reduced expression of, β2-microglobulin and so of the HLA class I determinant protects a tumour from direct cytotoxic T-cell attack.[31] Consonant with this suggestion is the fact that the β2-microglobulin mutations largely occur in the sort of repeat sequences that are presumed to be susceptible to mismatch repair defects. In marked contrast, the APC mutations which are found in those sporadic tumours which have mismatch repair defects do not show, for example, an increase in the frequency of frameshift mutations strongly suggesting that in this case the APC mutations, as expected, occur before the mutation in one of the mismatch repair genes.[32] These data, together with theoretical considerations,[33] strongly imply that the mismatch repair mutations are not selected because of their effect on mutation rate, but presumably because of some other direct selective advantage. The clue to this puzzle comes from a consideration of the explanation for the high frequency of p53 mutations in many human cancers. These also increase genomic instability, but it is now clear that their selective effect is primarily on the frequency of programmed cell death or apoptosis.[34] Early stages in tumour progression, while increasing the independence and persistence of cell growth, may actually also increase the probability of cell death, leading therefore to very strong selection for mutations that counter this tendency. The p53 mutations, which in colorectal carcinomas occur after APC mutations-probably during the late adenoma, or early carcinoma stage[35,36]—fit in precisely with this explanation. A similar mechanism probably accounts for the selection of mismatch repair mutations, initially in the heterozygous state. This can explain why there is no parallel selection for excision repair mutations at the somatic level, which would otherwise be expected if mismatch repair mutations were selected for simply because of their effects on increasing the mutation rate.[37]

The high frequency of many carcinomas with chromosomal instability and excessive aneuploidy has been well known for many years. All the more striking therefore is the demonstration that colorectal tumours with mismatch repair mutations do not show this chromosomal instability.[38] p53 mutations are one explanation for chromosomal instability in the absence of mismatch repair mutations. However, there is clearly a significant group of tumours which have neither mismatch repair nor p53 mutations in which other genetic changes must account for their chromosomal instability. Recently, Cahill et al[39] have shown that some colorectal tumours have mutations in genes which control the relationship between mitotic cell division and chromosome segregation, and so which may begin to fill the gap in explaining the genetic basis of chromosomal instability in colorectal carcinomas. It seems most probable that, as in the case of DNA repair, so also for control of chromosome distribution at cell division, there must be a default option which targets a cell for apoptosis when there are too many mistakes in chromosome segregation. It seems likely therefore that the mutations discovered by Cahill et al[39] may, as is the case for p53 and the mismatch repair mutations, be selected for because they interfere with the apoptotic process and so give rise to a positive selective advantage. This is presumably the reason why cells carrying such mutations in fact survive in the face of extensive aneuploidy. It appears that

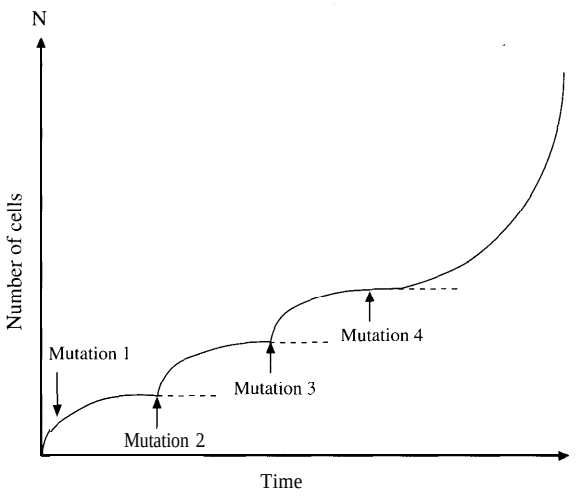


*Fig. 1. Successive mutation and selection steps for increasing levels of the number of intermediate proliferating zone cells to successive equilibria before onset of exponential growth.*

increased chromosomal instability, as in the case of increased mutation rates following either p53 or mismatch repair gene mutations, is a secondary effect in tumorigenesis which can, however, undoubtedly have profound consequences for the subsequent evolution of a cancer.

### Cell Division and Death: Models for the Cancer Process

There have been many attempts at formulating quantitative models for the cancer process. The age incidence curve for the onset of cancer, for example, provides a crude estimate of the number of steps that may be involved in giving rise to a cancer, based on a simple model of exponential growth.[40] Such models, however, consider only cell division and not cell death; do not take selection into account, and because they are based on exponential growth cannot easily explain the long lag period, often up to 20 years before colorectal carcinomas and other carcinomas develop, and also cannot account for benign tumours.

Population growth is a balance between birth and death rates, and this applies equally to the growth of a population of tumour cells. Thus, it is now recognised that the cell death rate, or rate of apoptosis, is as important if not more so in controlling the growth of a cancer as the death rate is in controlling any population growth. This is reflected in the frequency of p53 mutations found in human cancers and in the suggested basis for selection of mismatch repair and other instability-causing mutations. Indeed, it now seems that most current cancer therapy, either with X-rays or chemotherapy, induces apoptosis and so cell death by suicide rather than by direct killing.

A model for cancer progression which takes into account cell birth and death rates, as well as the probability of differentiation, using colorectal cancer as a template, has been derived by Tomlinson and Bodmer.[33] Colorectal crypts are clonal[41] and have at their base a stem cell population which divides and differentiates to give rise to the other cells of the crypt. The stem cells produce an intermediate proliferating zone of cells which in turn differentiates terminally before it reaches the luminal surface, dies by apoptosis and is shed into the lumen. A simple model for this process shows that mutations affecting either the death or differentiation rates of the intermediate proliferating population of cells often result in a new equilibrium between the number of stem cells, the number of intermediate proliferating cells, and the number of terminally differentiated cells that are shed into the lumen. This happens when the effects of the mutation rate are not too large.

Thus, a series of mutational steps can take place, which each time increase the number of intermediate cells relative to stem cells but do not yet lead to exponential growth and therefore frank carcinomas (Fig. 1). The more such steps have occurred, the higher the probability that the next change will lead to exponential growth and so to a cancer.

This model has major implications for understanding the nature of tumour progression. First of all, it explains the occurrence of a benign phase which is associated with the finite step increases in size in the intermediate

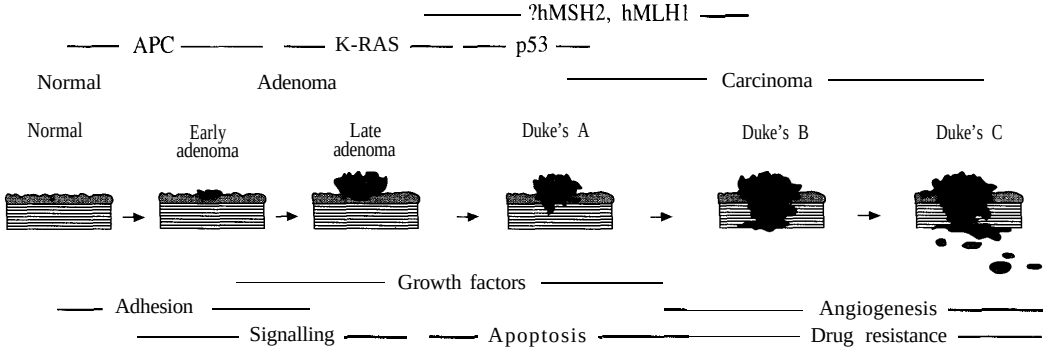


*Fig. 2. A scheme for the adenoma to carcinoma progression, indicating, above, the stages at which mutation in the APC, K-ras, p53, hMLH1 and hMLH1 genes occur. and below, the key functions being selectedfor at different stages.*

proliferating zone of cells. Only if mutations eventually arise which lead to exponential growth does the adenoma become an adenocarcinoma. Secondly, it can account for the long-lag phases before a cancer arises, without the awkwardness of having very low rates of exponential growth which are extremely hard to conceive over periods of up to 20 years, given the rapid rate of cellular turnover in the gut. By contrast, at each new plateau in the stepwise model, a considerable period of time may elapse before the next mutation arises and takes a hold and so provides the selective basis for a further increase in tumour size. The population of cells is at no time static, but is continually turning over at a rate of perhaps 100 cell generations per year. This provides extensive opportunity for the production of appropriate mutations with the selective advantage necessary to take a tumour to its next stage. Tumours which remain benign have either followed a deadend pathway with respect to the possibility of exponential growth, or simply have not yet had the chance to produce enough mutations to take them to the exponential growth phase.

## A Framework for Understanding Cancer

The data and ideas on the somatic evolution of cancer discussed above in the context of colorectal cancer, but applicable to many if not most other cancers, now provide a basic framework for understanding the initiation and progression, at least of epithelial tumours (Fig. 2). Mutations occur in a series of pathways that are critical for different stages of tumour initiation and progression. Thus, for example, since the earliest mutation appears to be associated with the APC gene and pathways involving the control of cellular attachment, this suggests that this is the first pathway in which mutations may be found for most carcinomas. This is consistent with finding β-catenin mutations, especially in cancers that do not have APC mutations, and with finding E-cadherin mutations in certain other cancers.[42,43] Subsequent pathways involve signalling, perhaps in relation to growth control by growth factors, which accounts for the K-ras mutations. Further mutations presumably may be found in other signalling molecules, and in growth factors or their receptors as has been found in other cancers. Another absolutely critical pathway, as already extensively emphasised, is that involving the control of apoptosis. This involves mutations in p53, the mismatch repair genes, and in genes controlling chromosome stability as well as in other cancers, other aspects of cell cycle control. Later changes in cell surface and growth properties then presumably promote metastasis and the angiogenesis that is needed to feed larger primary and also secondary growths. Later genetic changes lead to resistance to naturally developed anticancer immunity as well as to therapy, especially using cytotoxic drugs or, for example, hormone antagonists. The early disruption of tissues associated with changes in adhesive properties of epithelial cells, may be enough to give the equivalent of an inflammatory response to promote initial vascularisation that can sustain the early stages of tumour growth. That is why strong selection for angiogenesis may be a somewhat later event.

Each new genetic step discovered provides a new opportunity for the development of assays to identify more specific drugs for the treatment of cancer and potential new targets for immunotherapy. New improved mouse models for human cancers, based on the rapidly developing genetic understanding, will furthermore provide a more effective basis for exploring dietary and environmental factors in the genesis of cancer as well as for testing new forms of therapy.

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